THE BEHAVIOR *of* DEAMINIZED GELATIN

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DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
THE FACULTY OF PURE SCIENCE, COLUMBIA
UNIVERSITY IN THE CITY OF NEW YORK

BY

ZANVIL CHARLES LOEBEL, B.S., M.A.

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To

My Father and Mother

ACKNOWLEDGMENT

This investigation was undertaken at the suggestion of Professor Arthur W. Thomas. It is a pleasure to express sincere appreciation of his kindly interest and advice throughout the course of the work.

THE BEHAVIOR OF DEAMINIZED GELATIN

Recent investigations, especially those of Proctor, of Wilson and of Loeb[1], have shown the importance of the study of the physical chemistry of the proteins as a function of the hydrogen ion concentration. These three workers have demonstrated that at least four properties of the proteins (osmotic pressure, swelling, viscosity and membrane potentials) can be explained by the Donnan Theory of Membrane Equilibria. This theory, briefly stated, holds that when a membrane separates two solutions of electrolytes, one of which contains non-diffusible ion while the other ions are diffusible, the result will be an unequal distribution of the diffusible ions on the opposite sides of the membrane. This unequal distribution of the diffusible ions gives rise to potential differences and osmotic forces, which it is postulated, control the colloidal behavior of proteins.

According to the modern view, a protein is an amphoteric electrolyte capable of combining with hydrogen iron at the terminal amino groups and at the polypeptid linkages; and capable of neutralizing hydroxyl ion at the terminal carboxyl groups and at the polypeptid linkages. The hydrogen ion concentration at which a protein is electrically neutral is called its iso-electric point. At this reaction it is as acidic as it is basic.

The study of many of the properties of gelatin as a function of hydrogent ion concentration indicate a transition point at its iso-electric point, pH 4.7. Thus at pH 4.7 there is a minimum of solubility, osmotic pressure, swelling, viscosity at 25° C, potential difference, optical rotatory power at and above 27½° C, and absorption of light; a maximum is found at this point for foaming and optical rotation below 27½° C. Because of the poor buffering action of gelatin in the range from about pH 7.0 to about pH 8.7 few measurements were made in this region in the earlier investigations. In 1922 Wilson and Kern[2] found that the swell-

ing of buffered gelatin solutions gave a swelling-pH curve with a second minimum at pH 7.7. A second transition point has been found for other properties too; and the work of Davis and Oakes[4] indicate a shift of the minimum for viscosity at 40° C from pH 4.7 at 25° C to about 8.0.

So far as is now known, there are only three proteins which show a second transition point in their properties-pH curve. They are collagen (the mother substance of gelatin), deaminized collagen and gelatin. C. R. Smith[5] points out that gelatin is the only protein which shows optical mutarotation and that as the power to jellify is lost—such as by prolonged heat treatment—the ability to mutarotate disappears too.

It was thought that an investigation of the behavior of gelatin, on whose molecular structure the free amino groups had been replaced by less reactive groups might throw further light on the physical chemistry of the proteins. Such an alteration of the structure of the gelatin molecule should change its chemical and physical properties in a definite and measureable manner.

The results are discussed in the following order:

(1) The preparation of deaminized gelatin
(2) Determination of the iso-electric point by the dye technique
(3) The optical rotatory power of deaminized gelatin
(4) Viscosity of deaminized gelatin, in which are included viscosity experiments with ordinary gelatin
(5) Titration of gelatin and deaminized gelatin with sodium hydroxide
(6) Surface tension and foaming of deaminized gelatin
(7) Discussion of the second transition point

Materials Used

In all the experiments "Putmann's Silver Label Gelatin" was used as a source of ordinary and deaminized gelatin.

The sodium nitrite, glacial acetic acid, ammonium sulphate, sodium hydroxide and hydrochloric acid used were of the ordinary C.P. variety and the two dyes for the iso-electric point experiment were used as received from the manufacturer.

The Preparation of Deaminized Gelatin*

Introductory

When a protein such as gelatin is treated with nitrous acid there is an evolution of nitrogen, presumably from the reaction of the nitrous acid with the amino groups of the protein, the amino nitrogen being replaced by hydroxy groups. This reaction forms the basis of the Van Slyke method for the quantitative estimation of amino nitrogen. Nitrous acid was first used as a deaminizing agent for proteins in 1885 by Loew[6]. Skraup[7] and his co-workers have been largely responsible for perfecting the methods used in preparing deaminized proteins. They added glacial acetic acid to a solution of the protein and sodium nitrite, and after allowing the mixture to remain over-night, warmed it on a water-bath for two hours; then solid ammonium sulphate was added, yielding a bright yellow resin-like product which was drained off in linen, desiccated and dried in air.

In 1914 Blasel and Batula[8] deaminized gelatin according to Skraup's method and showed by hydrogen electrode measurements that the deaminized protein was still capable of combining with hydrochloric acid. Recently Hitchcock[9] has published a quantitative study of the acid combining capacity of ordinary and deaminized gelatin. He prepared the deaminized product by Skraup's method, but omitted the heating on the water-bath, a preliminary study indicating that the higher temperature induced a slight hydrolysis. Analysis of Hitchcock's preparation showed an exact agreement between the difference of the total nitrogen for ordinary and deaminized gelatin, and the amino nitrogen removed in the Van Slyke analysis; in each case 0.00040 equivalents of nitrogen per gram. Furthermore, the difference between the maximum combining capacity of the ordinary and the deaminized products for hydrochloric acid was nearly equivalent to the loss in amino or total nitrogen in the deaminization.

Procedure

The deaminized gelatin was prepared after Hitchcock's method: 100 gms. of gelatin was dissolved in one liter of warm

* The term "deaminized gelatin" as hereinafter used, refers to the gelatin which has undergone the nitrous acid treatment as described in this section.

water and cooled. A solution of 100 gms. of sodium nitrite in 50 cc of water was added, after which 70 gms. of glacial acetic acid was gradually added with continuous stirring to expedite the removal of gases. At the end of twenty-four hours solid ammonium sulphate* was added to complete precipitation of a bright yellow resin-like mass. This mass was found to swell in cold water like gelatin. It was placed in linen sacs, which in turn were placed in dark bottle and cold tap water was run through for 24 hours. All washing was carried out in a dark box, as strong light changes the color to a reddish-brown. Repeated washing with cold water brought to pH 4.0 by addition of hydrochloric acid to distilled water, followed by repeated washing with cold distilled water; and finally dehydration with 95 per cent alcohol and then absolute alcohol gave a bright yellow fibrous solid. This was ground in a pebble mill for 24 hours and passed through a 60 mesh sieve. The final product was left exposed to the atmosphere for a day to allow it to reach equilibrium with atmospheric moisture.

Analysis of the final product for total nitrogen by the Kjeldahl method gave 17.38 per cent while the original gelatin contained 17.96 per cent; a loss of .58 per cent or 4.1×10^{-4} equivalents per gram absolutely dry weight.

Ordinary iso-electric, ash-free gelatin was prepared according to the method of Loeb[1]. The gelatin was repeatedly washed with cold water brought to pH 4.7 by addition of acetic acid, then with cold distilled water and dehydrated with 95 per cent and finally absolute alcohol. The product was ground in a pebble mill for 24 hours, passed through a 60 mesh sieve and left exposed to the atmosphere for a day to reach equilibrium with the atmospheric moisture.

The moisture in all samples of ordinary and deaminized gelatin was determined by heating in a 105° C oven over-night and all weights are reported on this moisture-free basis.

Method of Effecting Solutions for Optical Rotation and Viscosity Experiments

It was found that because of the color and the turbidity of the solutions of a concentration below 0.5 per cent would be

* When solid sodium chloride was used in place of the ammonium sulphate the resin-like mass was less yellow at first and on exposure to strong light became a greenish-brown.

necessary in order to make optical rotation readings. It was planned to compare the different properties at a constant concentration and after a preliminary study, 0.4760 per cent was found most satisfactory.

The solutions were made up as follows: To 0.4760 gms. of sample in a 100 cc. standard flask, 10 cc. of distilled water was added, then varying amounts of acid or alkali and finally enough distilled water to reach the 100 cc. mark. After remaining at room temperature for one hour to allow the sample to swell, the flask was placed in a water-bath at either 50° C or at 75° C for five minutes, removed, inverted slowly five times and replaced in the same bath for fifteen minutes. The flask was then kept in a 25° C water-bath for ten minutes and was ready for use.

All pH values recorded in this report were obtained with the aid of a saturated KCl-calomel half cell junction with the solution being effected through a saturated salt bridge. pH was calculated from the equation, $pH = \frac{E-0.2466}{0.0001987T}$. Measurements were made at room temperature, which was recorded in each case.

Determination of the Iso-Electric Point of Deaminized Gelatin by the Dye Technique

It will be recalled that Loeb[1] has shown that protein can combine with cations only on the alkaline side of its iso-electric point, and with anions only on the acid side. Hence deaminized gelatin should combine with the colored cation of a basic dye on the alkaline side, and the colored anion of an acid dye on the acid side of its iso-electric point. The iso-electric point as determined by the dye technique for gelatin, collagen and deaminized collagen show good agreement with the values obtained by other methods.

Procedure

Portions of deaminized gelatin equal to 1 gm. were placed in two series of beakers and 50 cc. of acetate buffer solution of varying hydrogen ion oncentration were added, and then kept in the ice-box for two hours, being stirred frequently. These were filtered on Gooch funnels fitted with a cotton plug and two successive 20 cc. portions of dye solution passed through. The acid

dye, Acid Black, was used for one series, and the basic dye, Fuchsin, was used for the other series. The dye solutions contained 1 gm. of dyestuff per liter of solution.

After the dye solutions drained through, each portion of deaminized gelatin in the Gooch funnels was repeatedly washed with equal parts (alternated) of cold water and the respective buffers, and finally with the buffer only, until definite observations could be made. The results are given in Table I.

TABLE I.

Determination of Iso-Electric Point of Deaminized Gelatin

Acid Black	Fuchsin	pH of Solution
Deep Blue	*Yellow	3.6
**Green	*Yellow	3.8
**Green (slight)	Reddish	4.0
*Yellow	Red (deep)	4.2

These results indicate the iso-electric point to be at pH 4.0, coinciding with the value obtained by Hitchcock[9] for minimum of osmotic pressure.

Optical Rotation of Deaminized Gelatin

C. R. Smith[5] postulated that there is a close relationship between the mutarotation of gelatin and its power to jellify. Prolonged heating on a water-bath causes a gelatin solution to lose both its power to jellify and to mutarotate. Kraemer and Fanselow[12] studied the optical rotation of gelatin solution of varying hydrogen ion concentration at different temperatures, 10° C, 15° C, 20° C, 25° C, 27½° C, 30° C, 35° C, 40° C, and 50° C,—using the mercury light as a source of illumination. Their results of optical rotation as a function of pH show a minimum both at pH 4.7 and at about pH 8.0 for temperatures of 27½° C and above; and show a maximum at the same points for temperatures below 27½° C.

It was planned to study the optical properties of deaminized gelatin and to compare them with those of ordinary gelatin.

*Color of deaminized gelatin.
**Due to yellow of deaminized gelatin plus the blue of the dye.

Procedure

The solutions for the optical rotation experiments were prepared as described above, the solution being effected at 50° C. The solutions were polarized in 2.2 dcm, jacketed tubes, water respectively at 10° C, 25° C, and 50° C (±.3 C) being pumped through the jackets. After 15 minutes at the proper temperatures, readings were taken. The instrument used was a Schmidt and Haensch saccharimeter, with a dichromate filter. White light was used as a source of illumination. The direct readings (degrees Ventzke) at 10° C, 25° C and 50° C are tabulated in Table II and plotted as a function of pH in Figure I.

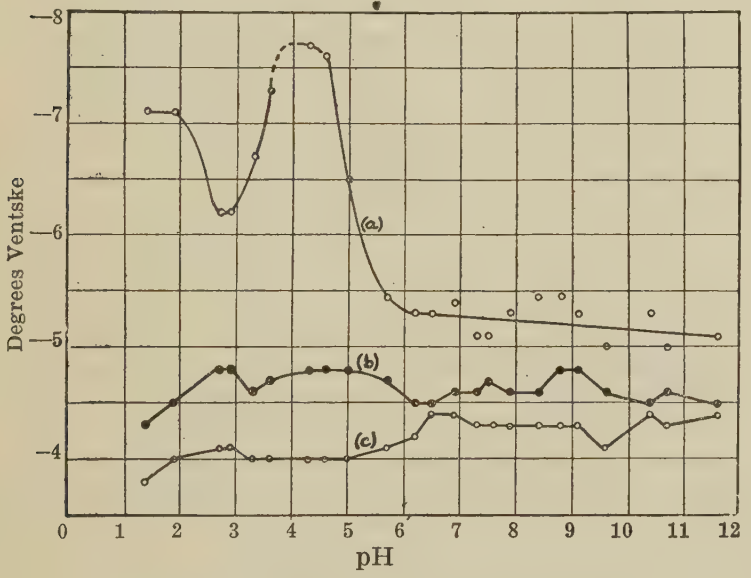

Fig. I.—Effect of temperature and pH on the optical rotation of .4760 per cent solution of deaminized gelatin.

(a)=10°C
(b)=25°C
(c)=50°C

TABLE II.
Optical Rotation of Deaminized Gelatin at Varying pH and Temperatures

pH of Solutions	Negative Ventzke Reading at 10° C	25° C	50° C
1.4	7.1	4.3	3.8
1.9	7.1	4.5	4.0
2.7	6.2	4.8	4.1
2.9	6.2	4.8	4.1
3.3	6.7	4.6	4.0
3.6	7.3	4.7	4.0
3.9	turbid	turbid	turbid
4.0	"	"	"
4.3	7.7	4.8	4.0
4.6	7.6	4.8	4.0
5.0	6.5	4.8	4.0
5.7	5.4	4.7	4.1
6.2	5.3	4.5	4.2
6.5	5.3	4.5	4.4
6.9	5.4	4.6	4.4
7.3	5.1	4.6	4.3
7.6	5.1	4.7	4.3
7.9	5.3	4.6	4.3
8.4	5.2	4.6	4.3
8.8	5.2	4.8	4.3
9.1	5.3	4.8	4.3
9.6	5.0	4.6	4.1
10.4	5.3	4.5	4.4
10.7	5.0	4.6	4.3
11.6	5.1	4.5	4.4

Because of the turbidity and color no readings could be made at pH 4.0. The curve for 10° C readings shows a minimum at pH 2.9 and a tendency to form a maximum as the pH approaches 4.0. On the alkaline side of pH 4.0 the rotation falls off with increasing pH. At 25° C and 50° C the rotation is almost constant for each respective temperature. Unfortunately, the color and turbidity do not permit the use of higher concentrations which might magnify any deviations, and detect any mutarotation.

Viscosity of Deaminized Gelatin

It will be recalled that Loeb's[1] viscosity-pH curves of gelatin at 25° C indicate a minimum of viscosity at the iso-electric point, pH 4.7. The viscosity-pH curves of Davis, Oakes and Brown[3] at 25° C indicate no minimum at pH 4.7, while the viscosity-pH curves of Davis and Oakes[4] at 40° C give no minimum at 4.7 but give one at about 8.0. Loeb's solutions were effected by heating to 45° C for ten minutes. Hitchcock[14] found the viscosity-pH

curve of gelatin at 40° C (solution effected by heating to 40° C), to be similar to that obtained by Loeb, with a minimum at pH 4.7.

Blasel and Matula[8] measured the viscosity of deaminized gelatin but no valid conclusions can be drawn from their results because their observations were made at such wide intervals of pH.

Two groups of experiments on viscosity of deaminized gelatin were performed; in the first the solution was effected at 50° C and in the second the solution was effected at 75° C. In the first group, 5 cc of each solution was pipetted into three Ostwald viscosimeters which were placed, respectively, into water-baths regulated to 10° C, 25° C and 50° C (±.1 C), after fifteen minutes the viscosities were measured. In the second the same procedure was followed except that the determinations at 10° C, were omitted. The results are given in TABLES III and IV and plotted in FIGURE II. The viscosities are reported as "Viscosity Ratio", V:

$$V_t = \frac{\text{Time in seconds at temperature t for deaminized gelatin solution}}{\text{Time in seconds at 25° C for water}}$$

TABLE III.

Viscosity of Deaminized Gelatin Solutions at Varying Temperatures and Hydrogen Ion Concentrations

Batch No. 3
Concentration of deaminized gelatin 0.4760%.
Solution effected at 50° C.

pH of Solution	$V_{10°\,C}$	$V_{25°\,C}$	$V_{50°\,C}$
1.4	2.695	1.312	.7915
1.9	2.746	1.542	.9123
2.7	3.222	1.791	1.036
2.9	3.316	1.836	1.050
3.3	3.199	1.654	.9538
3.6	2.871	1.452	.8578
3.9	2.692	1.217	.7251
4.0	turbid	turbid	turbid
4.3	2.866	1.240	.7275
4.6	3.104	1.427	.8282
5.0	3.457	1.775	1.055
5.7	3.765	2.309	1.322
6.2	3.800	2.396	1.456
6.5	3.849	2.448	1.506
6.9	3.801	2.435	1.436
7.3	3.770	2.406	1.403
7.6	3.771	2.418	1.417
7.9	3.774	2.442	1.421
8.4	3.698	2.397	1.405
8.8	3.511	2.239	1.334
9.1	3.365	2.127	1.256
9.6	3.207	2.045	1.175
10.4	3.055	1.970	1.120
10.7	2.968	1.958	1.118
11.6	2.384	1.440	.8863

TABLE IV

Viscosity of Deaminized Gelatin Solutions at Varying Temperatures and Hydrogen Ion Concentrations

Batch No. 4.

Concentration of deaminized gelatin 0.4760%.

Solution effected at 75° C.

pH of Solutions	$V_{25°C}$	$V_{50°C}$
1.5	1.198	.7251
2.3	1.357	.8128
2.9	1.411	.8389
3.3	1.367	.8199
3.7	1.264	.7465
4.0	1.179	.7038
4.4	1.269	.7276
4.7	1.361	.7998
5.5	1.787	1.066
5.9	1.872	1.113
6.2	1.987	1.190
6.5	2.002	1.199
7.0	1.978	1.185
7.3	1.965	1.169
7.4	1.995	1.182
7.9	2.000	1.185
8.3	1.933	1.175
9.2	1.861	1.118
9.9	1.754	1.050
10.3	1.752	1.051
11.0	1.520	.9123

Although turbidity at pH 4.0 of solutions effected at 50° C permitted no viscosity measurements at this point, the direction of the curves on both sides show a distinct tendency to locate the minimum at this point.

It will be noted further that:

(a) Each curve, whether the solutions were effected at a 50° C or 75° C, or whether the viscosities were determined at 10° C, 25° C or 50° C is similar in shape.

(b) Each has a minimum at its iso-electric point, pH 4.0.

(c) In each the rise on the alkaline side is greater than on the acid side.

(d) Each has a second minimum at pH 7.3.

(e) Each has maximums at pH 2.9, and 7.9.

In TABLE V and FIGURE II below are given the results of a series of experiments with gelatin solutions at 25° C and 50° C gelatin described above (0.4760%). The solutions were effected

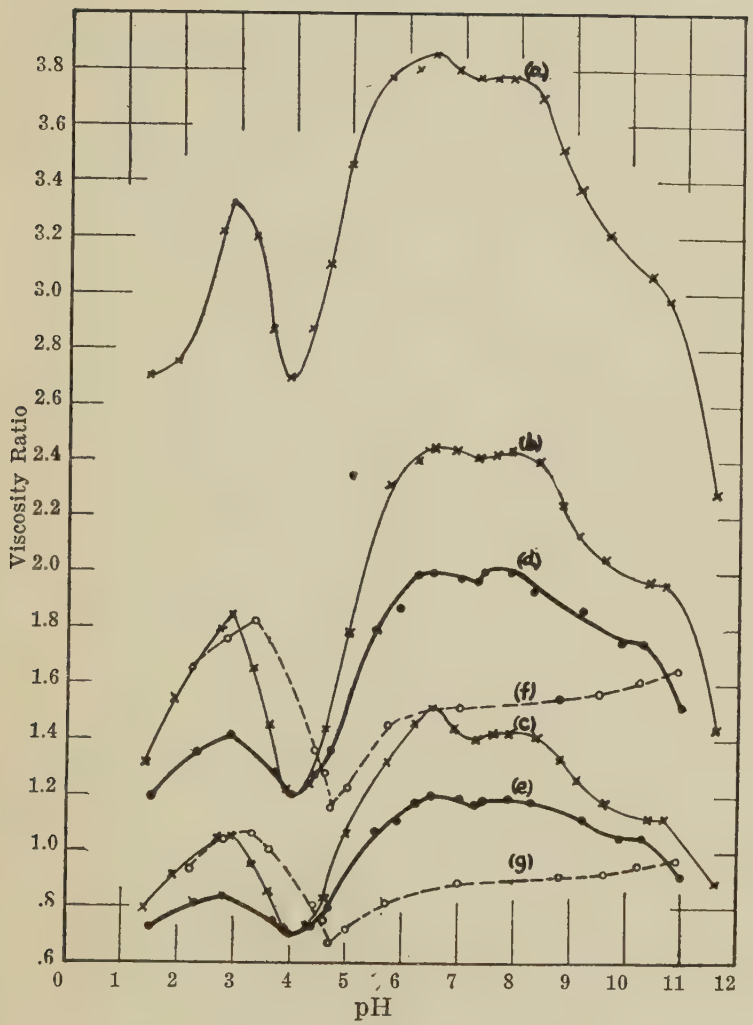

FIG. II.—.4760% solutions of:

†Deaminized Gelatin—Solution effected at 50°C. Viscosity at. (a) 10°C, (b) 25°C, (c) 50°C

*Deaminized Gelatin—Solution effected at 75°C. Viscosity at (d) 25† C, (e) 50°C

°Gelatin—Solution effected at 75°C. Viscosity at (f) 25°C, (g) 50°C

at 75° C, the same technique as used for the viscosities described being employed. These curves and the curves found by Loeb at

25° C and by Hitchcock at 40° C show many points of similarity with those of deaminized gelatin:

(a) Each of the gelatin curves is similar in shape, independent of temperature of effecting the solution.

(b) Each has a minimum at the iso-electric point. In the deaminizing reaction amino groups are replaced by less basic hydroxy groups, and it is thus expected that with deaminization there should be a shift of the iso-electric point, as does occur.

TABLE V.

Viscosity of Gelatin Solutions at Varying Temperatures and Hydrogen Ion Concentrations

0.4760% gelatin solutions.
Solutions effected at 75° C.

pH of Solutions	$V_{25°\,C}$	$V_{50°C}$
2.2	1.653	.9348
2.8	1.759	1.041
3.3	1.820	1.062
3.6	1.749	1.005
4.4	1.361	.8057
4.6	1.282	.7500
4.7	1.157	.6718
5.0	1.226	.7204
5.7	1.446	.8152
7.0	1.517	.8898
8.8	1.549	.9135
9.6	1.565	.9240
10.2	1.612	.9502
10.9	1.646	.9739

(c) With gelatin the rise on the acid side of the iso-electric point is greater than that on the alkaline side; the reverse is the case with the deaminized gelatin. Comparing the 25° C and 50° C curves of FIGURES III and IV it is seen that deaminization does not effect the viscosity at the iso-electric point, but lowers it on the acid side and raises it on the alkaline side; the rise on the alkaline side being very steep.

Thomas and Foster[15] report a similar steeper rise of swelling with deaminization of collagen. They suggest as a possible explanation that the hydroxy groups which have substituted the amino groups are acidic in character. This would result in the formation of a greater amount of sodium salt on addition of sodium hydroxide and thus cause greater swelling.

The same explanation may be applied to account for the anomalous behavior of viscosity with deaminization of gelatin. Einstein's theory[1] holds that the viscosity is a linear function of the relative volume occupied by solute in solution. The volume

size of the protein solute, according to Loeb, would be dependent upon the swelling and osmotic forces which are governed by the Donnan Theory. It has been shown by Blasal and Matula[5] and by Hitchcock[9], that deaminized gelatin combines with less hydrochloric acid than does gelatin. Then following Loeb's theory, deaminized gelatin should have a lower viscosity than gelatin on the acid side, as is actually the case. In a later section a greater base-combining capacity is shown for the deaminized product, thus accounting for the greater viscosity on the alkaline side.

(d) The maximum on the acid side of the iso-electric point for gelatin is at pH 3.2, while that for the deaminized is at pH 2.9, indicating again a shift to the acid side.

(e) Because of the poor buffering of gelatin in the pH range from about 7.0 to 8.7, neither Loeb nor Hitchcock made measurements in this region. However, a study of other properties indicates a second minimum at pH 7.7. The second minimum for deaminized gelatin is at pH 7.3 showing still another shift towards the acid side. A more complete discussion of the second transition point must be left to a later section.

Titration of Gelatin and Deaminized Gelatin with Sodium Hydroxide

In the preceding section it was pointed out that the increase of viscosity on the alkaline side of the iso-electric point with deaminization is very likely due to the acidic nature of the hydroxy groups[15] that replaced the amino groups. If this is true the base-combining capacity of deaminized gelatin should be greater than that of gelatin by an amount equivalent to the replacing hydroxy groups. Because of the steep rise in viscosity on the alkaline side beginning at the iso-electric point it was expected that the titration curve of the deaminized gelatin with sodium hydroxide would be very steep beginning at pH 4.0; i.e., the replacing hydroxy groups would begin to react with sodium hydroxide just above pH 4.0.

To obtain comparable results both gelatin and deaminized gelatin were titrated. Samples equal to 0.4617 gm. absolutely dry weight were used; varying amounts of 0.1012N sodium hydroxide added and the pH determined electrometrically. A single sample was employed for each titration curve, the original volume

being 25.30 cc. Preliminary results indicated that to adjust the pH of gelatin and deaminized gelatin to pH 7.0 required respectively 1.43 cc. and 3.01 cc. of the sodium hydroxide used. To keep the volumes at pH values above 7.0 more nearly constant, 1.58 cc. (the difference between 3.01 and 1.43) of water was added to the gelatin sample.

The results are given in Tables VI and plotted in Figue III.

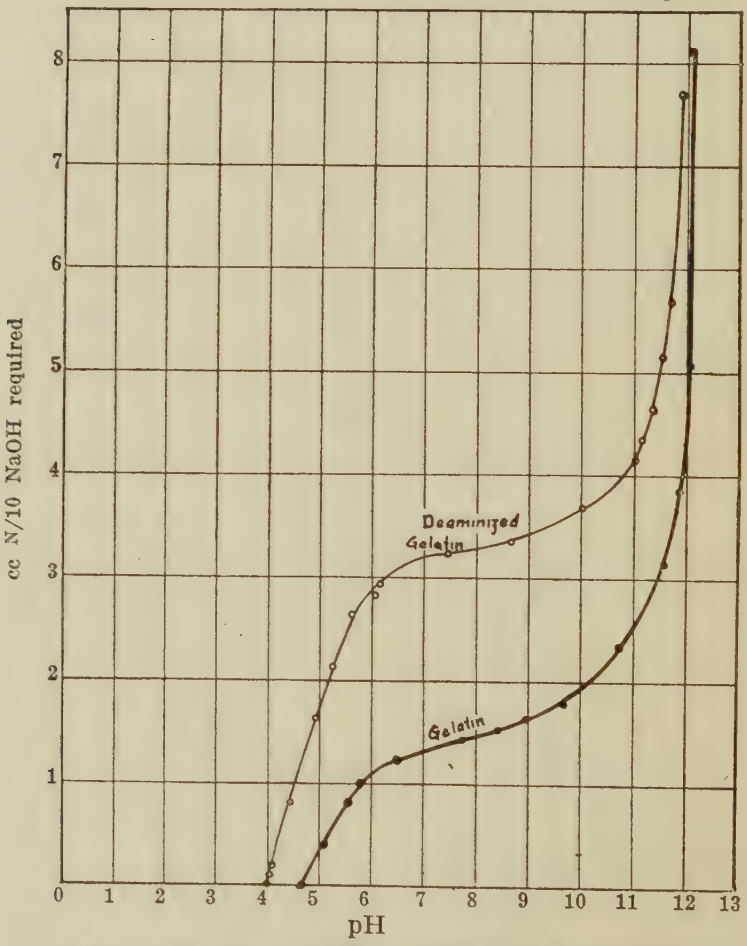

FIG. III.—Titration Curves for .4617 gms of originally isoelectric: •Gelatin
and
°Deaminized Gelatin
with N/10 NaOH.

TABLE VI

Titration of Gelatin with Sodium Hydoxide

Weight in gelatin sample 0.4617 gm. (dry weight).

Na OH Required* cc .1N	E.M.F. Volts	pH
0.0	.5216	4.70
.40	.5457	5.11
.81	.5721	5.56
1.01	.5843	5.77
1.21	.6274	6.51
1.42	.6996	7.74
1.52	.7406	8.44
1.62	.8722	8.98
1.77	.8737	9.69
2.33	.8755	10.74
3.14	.9231	11.56
3.85	.9412	11.86
5.06	.9500	12.01
8.10	.9543	12.09

TABLE VII.

Titration of Deaminized Gelatin with Sodium Hydroxide

Weight in gelatin sample 0.4617 gm. (dry weight)

Na OH Rquired** cc .1N	E.M.F. Volts	pH
0.0	.4806	4.00
.10	.4834	4.05
.20	.4860	4.09
.81	.5052	4.42
1.63	.5340	4.91
2.12	.5533	5.24
2.64	.5745	5.60
2.83	.6010	6.06
2.93	.6065	6.15
3.24	.6718	7.45
3.35	.7540	8.67
3.69	.8330	10.02
4.15	.8912	11.02
4.35	.8993	11.15
4.65	.9100	11.34
5.15	.9202	11.51
5.67	.9311	11.70
7.69	.9421	11.89
10.73	.9501	12.02
14.77	.9561	12.13

*cc of exactly .1N required to adjust pH from 4.70 to that recorded in the last column.

The pH of the gelatin dissolved in water was 4.58. It was necessary to add .15 cc of the 0.1012 N alkali to bring it to pH 4.70.

**cc of exactly .1N required to adjust pH from 4.00 to that recorded in the last column.

The deaminized gelatin sample dissolved in water gave a pH of 4.05. To bring the pH to 4.00 it was necessary to add .10 cc of .1N acid.

(a) Examining the slopes of the first part of each curve, rising from their respective iso-electric points, it is seen that the slope of the curve for the deaminized sample is steeper.

(b) This first portion of the curve for the deaminized gelatin rises to a higher value than that of the gelatin indicating that the hydroxy groups begin to combine with sodium hydroxide directly beyond the iso-electric point. The sudden rise in viscosity directly beyond the iso-electric point is thus accounted for.

(c) At pH 7.0:

0.4617 gm of deaminized gelatin combines with 3.15 cc .1N sodium hydroxide.

0.4617 gm of gelatin combines with 1.30 cc .1N sodium hydroxide.

The difference is 1.85 cc .1N sodium hydroxide.

For 1 gm of the protein this difference is 4.0 cc .1N sodium hydroxide or 4.0×10^{-4} equivalents per gm.

(d) There is a sudden change of slope at pH 7.5 for the deaminized gelatin which corresponds to a similar point for the gelatin at pH 7.7; it is interesting that pH 7.7 corresponds to the "second transition point" of the properties of gelatin.

At pH 7.5—0.4617 gm of deaminized gelatin combines with 3.24 cc .1N sodium hydroxide.

At pH 7.7—0.4617 gm of gelatin combines with 1.40 cc .1N sodium hydroxide.

The difference is 1.84 cc .1N sodium hydroxide.

For 1 gm this difference is 4.0 cc .1N sodium hydroxide or 4.0×10^{-4} equivalents.

The Kjeldahl nitrogen of the:

Gelatin	17.96%	
Deaminized gelatin	17.38%	
Difference	.58%	or 4.1×10^{-4}

equivalents nitrogen per gm. showing very good agreement with the difference of their base-combining capacity.

(e) If the curves are examined beyond pH 7.7 it is seen that for the same pH the difference in cc sodium hydroxide is prac-

tically constant, and it seems, therefore, safe to assume that the base-combining capacity of deaminized gelatin is equal to that of gelatin plus 4.0 x 10^{-4}. Taking the value of the base-combining capacity of gelatin as found by Loeb[11] and Hitchcock[10] at 5.7 x 10^{-4}, that for deaminized gelatin would be 9.7 x 10^{-4} equivalents per gm. absolutely dry substance.

Surface Tension and Foaming of Deaminized Gelatin

Like gelatin, deaminized gelatin solutions foam on shaking. The adsorption film theory advanced by Bancroft is founded on Gibbs' statement that any substance which lowers the surface tension of a liquid must concentrate in the surface, thus forming a film and preventing the coalescence to the gas bubbles. Accordingly, gelatin and deaminized gelatin should foam most at the points where the surface tension is least. Bogue[20] reports that the foaming of gelatin is a maximum at its iso-electric point. Johlin[26] finds its surface tension is a minimum at this point.

Surface Tension

Surface tension measurements were made with the DuNouy[27] tensiometer. The solutions, of 0.4760 per cent concentration, were made up by the same technique as described above, solution effected at 50° C. Two cc of each solution were pipetted into respective 4 cm watch crystals and left at room temperature for one hour. During this hour it was covered with an inverted Petri dish. Because of the exposed surface of the alkaline solutions it can not be claimed that the pH values are accurate. However, they are not sufficiently far removed to prevent the indication of the general direction of the surface tension change with pH change.

The usual precautions of flaming the pipette, watch crystals and platinum ring were taken. The apparatus was calibrated against water at 23° C giving the value of 73 dynes per cm. The results, given in TABLE VIII and plotted in FIGURE IV, indicate a minimum at pH 4.0, a maximum at pH 2.9 which remains constant with further decrease of pH; and a maximum at about pH 6.5, which remains constant with further increase of pH.

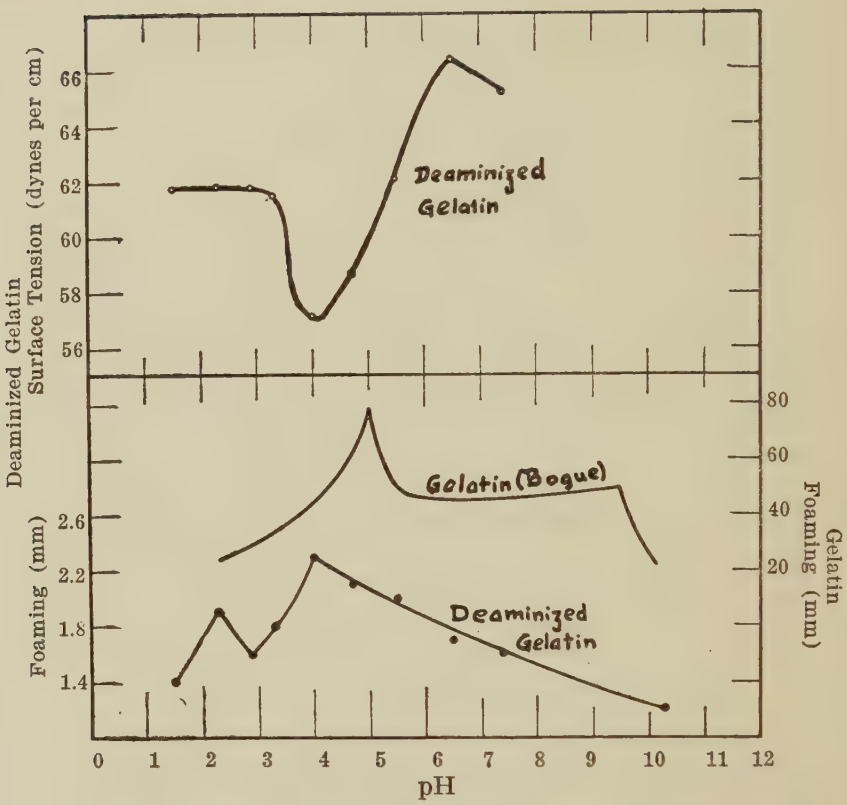

FIG. IV.—Effect of pH on the surface tension of .4760 per cent solution of deaminized gelatin and effect of pH on the foaming of .4760 per cent solutions of gelatin and deaminized gelatin.

TABLE VIII.

Surface Tension and Foaming of Deaminized Gelatin at Varying Hydrogen Ion Concentration

pH of Solutions	dynes/cm., at 23° C Surface Tension,	Foaming at 23° C Height in mm.
1.5	61.8	1.4
2.3	61.8	1.9
2.9	61.8	1.6
3.3	61.5	1.8
4.0	57.2	2.3
4.7	58.7	2.1
5.5	62.1	2.0
6.5	66.3	1.7
7.4	65.2	1.6
10.3		1.3

The foaming experiments were performed with the same solutions. Ten cc of each solution was added to respective 15 cc graduated test tubes and shaken all together, and the volume of foam read off. The results are recorded in TABLE VIII and plotted in FIGURE IV. They show a maximum at pH 4.0 and a minimum at pH 2.9. The curve obtained by Bogue is inserted for comparison. Although absolute values cannot be compared here the general trend of the two curves shows several interesting differences. Both curves show a maximum at their respective iso-electric points, but the gelatin curve has a second maximum on the alkaline side while the deaminized gelatin has the corresponding maximum on the acid side.

It will be noted that the surface tension in each case is lower than that of water and that the greatest lowering point of the surface tension curve is at the iso-electric point and corresponds with the highest point of foaming. These results are in conformity with the adsorption film theory, and are similar to those of Bogue and of Johlin for gelatin.

The Second Transition Point

Several properties of gelatin, studied as a function of the hydrogen ion concentration, give curves of the same type as the viscosity curves. Some of these properties have been studied in the unbuffered range. Wilson and Kern[2] found a second minimum for swelling of buffered gelatin at pH7.7. Higley and Matthews[16] found a second minimum at the same point for absorption of light. Kramer and Fanselow[12] and also Velles and Vellinger[13] found a second transition point for optical rotation.

There seems to be only four proteins which show these double transition points. They are collagen, deaminized collagen, gelatin and deaminized gelatin.

C. R. Smith[7] postulated two forms of gelatin in solution, a "sol" form stable at temperatures above 35° C and a "gel" form stable at temperatures below 15° C, while at temperatures between, the existence of both in equilibrium. The work of Smith and of Lloyd[17] show that the change from "gel" to "sol" form takes place with increase of Sörensen value of the solution. Wilson and Kern suggested that the two forms had different iso-

electric points, the "sol" form at pH 7.7 and the "gel" form at 4.7; and that a preponderance of either one would determine the behavior.

Support for this idea is found in the work of Thomas and Kelly[18], [19]. In studies of the rate of fixation of tannin by hide substances at room temperature[18] as a function of hydrogen ion concentration of the solutions they showed the degree of fixation at a maximum at pH 3 dropped to a minimum at pH 5 (the iso-electric point), rose again when a pH of about 8 was reached and then abruptly fell off with further increase in alkalinity. Since the tannin particles are negative, the decrease in rate of fixation from pH 3 to 5 was readily explained by the Proctor-Wilson theory as due to the decreasing positive charge of the collagen. The rise in rate of fixation from pH 5 to 8 was unexplainable except on the basis of a shift of the iso-electric point. Thomas and Kelly[19] repeating the same work at 40° C obtained no minimum at pH 5 and conclude, therefore, that the collagen exists in two modifications analogous to the "sol" and "gel" forms of gelatin; the iso-electric points of the collagen being at pH 5 and at 8.

With gelatin the evidence is more conflicting. To begin with, the exact location of the transition temperature, "gel" to "sol", is uncertain. C. R. Smith says it is at 35° C, Davis and Oakes place it at 38.03° C, and Kraemer and Fanslow place it at 27½° C. Bogue[20], on the other hand, says it depends on the concentration, heat treatment, pH and previous history.

Although Davis and Oakes report no minimum for the viscosity-pH curves of gelatin at pH 4.7 at 40° C and do report one at pH 8.0, it can not be said to represent a shift of the minimum due to the temperature. Davis, Oakes and Browne using the same technique as Davis and Oakes found the viscosity-pH curves of gelatin at 25° C gave no minimum at 4.7 either.

It was at first thought that the difference in method of effecting solution employed by Davis and his co-workers might be the cause for the difference in results. The matter was gone over with Dr. Davis and he was good enough to determine the viscosities of two series of gelatin solutions, one series being treated as in his two papers—by heating on a hot plate for 20 minutes with con-

tinuous stirring till the solution reached 70°; the other series being treated in a manner similar to that employed in this research. His results indicated that the same qualitative results were obtained by either treatment.

The interpretation that has been placed on the work of Davis et al has been that the minimum of viscosity at pH 4.7 at 25° C shifts to pH 8.0 at 40° C. Apparently the gelatin used by them did not have its iso-electric point at pH 4.7 as did that of Loeb, Hitchcock and that used in this research. Hitchcock's results for gelatin at 40° C and those reported in this investigation for gelatin and deaminized gelatin at various temperatures indicate no change of minimum with change of "gel" to "sol" form.

Loeb[1] postulated that the large changes of viscosity with pH of gelatin solutions at 25° C were due to two factors, the great magnitude of viscosity of gelatin solutions and the swollen micellae present. While such a theory is feasible enough for lower temperatures it can hardly hold for higher temperatures, like 50° C. However, as a matter of pure speculation, it might be suggested the viscosity of gelatin is dependent upon a solution structure[21] which in turn is dependent upon swelling forces and, therefore, according to the Donnan Theory, dependent upon osmotic forces.

In the viscosity experiments of gelatin and deaminized gelatin, the solutions of which were effected at 75° C, no shift of minimum was observed. A preliminary experiment in which gelatin solutions at different pH but in which the solution was effected by heating at 80° C for one hour indicated no shift of the minimum.

Although the above evidence indicates no shift of the minimum of gelatin and deaminized gelatin, there is every indication of the existence of the second point of inflection of properties. Wilson and Kern's idea of this point as a possible iso-electric point of the "sol" form finds further support in the work of Miss Lloyd[17]. She found that the "gel" —— "sol" equation, brought about with increase of pH is reversible if sufficient acid is added. She offers the following explanation: "Brailsford Robertson[22] has suggested that acids and bases attached themselves to protein molecules at the —COHN— linkage. He points out that this link-

age may exist as an enol linkage or as a keto linkage and favors the enol-form since it offers a point of attachment to both acids and bases. A more probable explanation seems to be that under the action of acids gelatin goes over to the keto-form, and under the action of bases to the enol-form." If this theory is correct all proteins should show the double transition points of properties. Although only four show this behavior, it seems that a more extensive study needs to be made to find a possible second point of inflection of other proteins.

The likelihood that the second point is a result of hydrolysis is small. Measurements by Bogue [20] and by Northrop [21] show that the rate of hydrolysis of gelatin at different pH is almost a constant for the same temperature. Northrop found the rate of hydrolysis is ten times more rapid at 65° C than at 40° C. It might be argued that the iso-electric point of these hydrolytic products is at pH 7.7. However, the experiments in this research indicated no shift of the minimum of viscosity with higher temperatures.

Knaggs and Schryver[25] conclude, after careful attempts in purifying gelatin to obtain a product of constant composition, that there is no reason for regarding gelatin, even after elaborate methods of purification, as a chemical entity. This suggests another possible cause for the presence of the two points of inflection as due to the predominating effect of first one group and then another group of substances present in the gelatin.

There seems to be an indication of a relationship between the chemical nature of gelatin and deaminized gelatin, and the second transition point of properties. The titration curve of gelatin with sodium hydroxide has a point of inflection at about pH 7.7, and that of deaminized gelatin at about 7.5; the former point corresponding to point of second minimum of swelling of gelatin as found by Wilson and Kern, and the latter almost corresponding to the second minimum of viscosity of deaminized gelatin as found in this research.

However, until more experimental work is done the cause for the existence of the second point of inflection must remain a matter of speculation.

SUMMARY

A study of the behavior of a deaminized gelatin has been made and compared with that of gelatin.

The iso-electric point of a deaminized gelatin was found by the dye technique at pH 4.0. The optical rotation, viscosity, surface tension and foaming of the solutions, was studied as a function of hydrogen ion concentration. The optical rotation at 10° C and the foaming at room temperature were at a maximum at the iso-electric point. Surface tension was at a maximum at this point. Whether the solutions were effected at 50° C or at 75° C, or whether the viscosities were determined at 10° C, 25° C or 50° C the viscosity-pH curve of deaminized gelatin was of the same shape, showing two minima, at pH 4.0 and pH 7.3.

Ordinary gelatin solutions effected at 75° C, gave viscosity-pH curves at 25° C and 50° C similar to those obtained by Loeb at 25° C, with a minimum at the iso-electric point.

Titration of deaminized gelatin and gelatin with sodium hydroxide indicated that the hydroxy groups which replace the amino groups in deaminization were acidic in character. The difference in the base-combining capacity of the two substances was equivalent to their difference in nitrogen content. The results indicated that the base-combining capacity of deaminized gelatin was 9.7×10^{-4} equivalents per gram.

The second point of transition of properties was discussed.

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VITA

Zanvil Charles Loebel was born in Newark, New Jersey, June 6, 1900. After graduating from the Polytechnic Institute of Brooklyn in 1922 with the degree of B.S. in Chemistry, he pursued graduate work in chemistry under the Faculty of Pure Science at Columbia, receiving the degree of M.A. in 1923. He was recipient of a "Special Soldiers, Sailors and Marines Scholarship" for the three academic years, 1921-1922, 1922-1923 and 1923-1924. During the academic year, 1923-1924, he was assistant in General Inorganic Chemistry at the Polytechnic Institute of Brooklyn. He is a member of the Society of Sigma Xi.